When Bridges Collapse: The Role of Disulfide Bridges in the Folding and Structure of Soybean Trypsin Inhibitor

J. Tetenbaum (Brandeis U.) and L. Miller (NSLS)

Abstract No. Tete2810
Beamline(s): **X19A, U10B**

Although it is well known that disulfide bridges stabilize the secondary structure of many proteins, little work has been done to determine the exact role that disulfide bridges play. In this work, the role of the two disulfide bonds in the structure and folding of Soybean Trypsin Inhibitor (STI) was investigated. The disulfide bonds were reduced with tris(2-carboxyethyl) phosphine (TCEP) at 40°C and the reduction process was probed using sulfur X-ray absorption spectroscopy. CD and FTIR spectroscopy were used concurrently to determine the structural changes caused by the reduction of the disulfide bridges. The non-cooperative disulfide reduction was completed within 5 minutes, likely because the disulfide bonds are located on the surface of the protein. The unfolding lagged behind; dramatic changes in secondary structure were not observed until 150-180 minutes after the reduction was initiated. The CD and FTIR spectra indicate a *decrease* in extended (hydrated) coil structure, contrary to expectations. Instead, the protein was observed to collapse, suggesting that the disulfide bonds in STI hold hydrophobic regions apart, preventing a hydrophobic collapse of the protein.

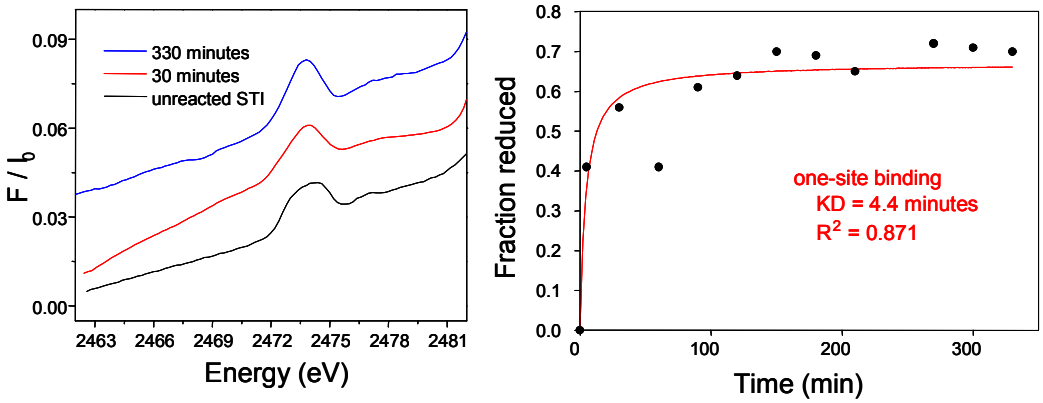

Figure 1. (*Left*) Sulfur X-ray absorption spectra of disulfide-intact STI and reduced STI 30 minutes and 330 minutes after the addition of tris (2-carboxyethyl) phosphine [TCEP] at 40ºC. (*Right*) Linear combinations of fully oxidized and fully reduced STI were used to determine the fraction reduced at each time point. Disulfide reduction in STI was found to be fast and non-cooperative.

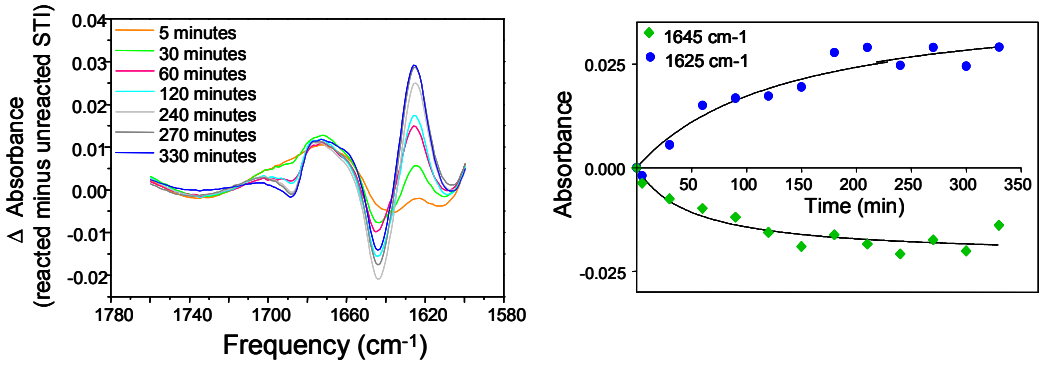

Figure 2. (*Left*) FTIR difference spectra at different time points during the reduction and unfolding of STI. (*Right*) The decrease in peak intensity at 1645 cm^{-1} suggests a loss of extended (hydrated) coil. The increase in peak intensity at 1625 cm^{-1} may be due to a collapse of the protein and an increase in hydrophobic interactions.